QTL mapping of partial resistance in winter wheat to *Stagonospora nodorum* blotch

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Abstract: *Stagonospora nodorum* blotch is an important foliar and glume disease in cereals. Inheritance of resistance in wheat appears to be quantitative. To date, breeding of partially resistant cultivars has been the only effective way to combat this pathogen. The partial resistance components, namely length of incubation period, disease severity, and length of latent period, were evaluated on a population of doubled haploids derived from a cross between the partially resistant *Triticum aestivum* 'Liwilla' and susceptible *Triticum aestivum* 'Begra'. Experiments were conducted in a controlled environment and the fifth leaf was examined. Molecular analyses were based on bulked segregant analyses using 240 microsatellite markers. Four QTLs were significantly associated with partial resistance components and were located on chromosomes 2B, 3B, 5B, and 5D. The percentage of phenotypic variance explained by a single QTL ranged from 14 to 21% for incubation period, from 16 to 37% for disease severity, and from 13 to 28% for latent period.

Key words: bulked segregant analysis, microsatellites, *Stagonospora nodorum*.

Résumé : Le *Stagonospora nodorum* est un agent pathogène qui cause la moucheture sur les feuilles et la tache des glumes, deux maladies importantes chez les céréales. La résistance chez le blé semble être de nature quantitative. À ce jour, la sélection de cultivars partiellement résistants était le seul moyen de lutte contre ce pathogène. Les composantes génétiques de cette résistance partielle, nommément la durée de la période d'incubation, la sévérité de la maladie et la longueur de la période de latence, ont été mesurées chez une population de lignées haploïdes doublées issues du croisement entre le cultivar partiellement résistant 'Liwilla' et le cultivar 'Begra' du *Triticum aestivum*. Les expériences ont été réalisées en milieu contrôlé et la cinquième feuille a été examinée. Des analyses moléculaires ont été effectuées à l'aide de 240 marqueurs microsatellites et en faisant appel à l'analyse des ségrégants regroupés (BSA). Quatre QTL montrant une association significative avec des composantes de la résistance ont été détectés sur les chromosomes 2B, 3B, 5B et 5D. La proportion de la variance phénotypique expliquée par un QTL variait entre 14 et 21 % pour la période d'incubation, de 16 à 37 % pour la sévérité de la maladie et de 13 à 28 % pour la période de latence.

Mots clés : analyse de ségrégants regroupés, microsatellites, *Stagonospora nodorum*.

[Traduit par la Rédaction]

Introduction

The fungus *Phaeosphaeria nodorum* (E. Müller) Hedjaroude syn. *Leptosphaeria nodorum* E. Müller (anamorph *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano syn. *Septoria nodorum* (Berk.) Berk.) is a foliar and glume pathogen of wheat (*Triticum aestivum* L.) and other cereals and grasses and is of world-wide economic importance (King et al. 1983; Scharen et al. 1985; Sprague 1950; Weber 1922). *Stagonospora nodorum* blotch causes crop yield loss and negatively impacts grain quality. The inheritance of resistance in bread wheat is usually quantitative and additive gene effects are most important (Bostwick et al. 1993; Ecker et al. 1989; Nelson and Gates 1982; Wicki et al. 1999; Wilkinson et al. 1990). However, a single gene for resistance to *S. nodorum* was reported in bread wheat (Frecha 1973; Kleijer et al. 1977), in *Triticum tauschii* (Murphy et al. 2000), and in durum lines with resistance derived from *Triticum timopheevii* (Ma and Hughes 1995). Several components of quantitative resistance have been investigated, including the area of the leaf infected, lesion number, infection frequency, lesion size, sporulation rate, incubation period, and latent period (Cunfer et al. 1988; Griffiths and Jones 1987; Jeger et al. 1983; Lancashire and Jones 1985; Loughman et al. 1996). Seedling and adult plant reactions are not always correlated (Mullaney et al. 1983), but adult plant resistance is frequently associated with increased plant height and late maturity (Rosielle and Brown 1980; Scott et al. 1982). Spike resistance to *S. nodorum* was reported to be independent of leaf resistance (Fried and Meister 1987) or at

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least partially controlled by different genes (Bostwick et al. 1993). Traditional breeding for such a complex quantitative trait based solely on phenotypic selection may be extremely difficult.

Marker-assisted selection could be more effective because molecular markers are not modified by the environment and can be detected at all stages of plant growth. Marker techniques based on the polymerase chain reaction (PCR; Mullis et al. 1986) greatly advanced genetic mapping of quantitatively inherited traits in wheat (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002). Microsatellites (Röder et al. 1998) are replacing other marker systems as the tool of choice, especially for gene mapping in wheat, because they detect a larger amount of DNA polymorphism (Bryan et al. 1997; Pestova et al. 2000*a*; Plaschke et al. 1995; Prasad et al. 2000; Stachel et al. 2000). Molecular markers linked to a trait of interest can be identified using bulked segregant analysis (Michelmore et al. 1991). Bulked segregant analysis (BSA), a method initially developed for mapping major genes, is also useful for mapping quantitative resistance loci in wheat (Bai at al. 1999; Chantret et al. 2000; William et al. 1997). Recently, molecular markers linked to single resistance genes to *Stagonospora nodorum* blotch were detected in bread wheat (Ellerbrook et al. 1999), in durum wheat (Cao et al. 2001), and in *T. tauschii* (Murphy et al. 1999). To our knowledge, there is only one study that explored resistance in *Triticum spelta* to *Stagonospora nodorum* using molecular markers in QTL analysis (Messmer et al. 1997).

The objective of our study was to detect QTLs associated with partial resistance components to *S. nodorum* in winter wheat. For mapping, we created a doubled-haploid (DH) population from a cross between resistant *T. aestivum* 'Liwilla' and susceptible *T. aestivum* 'Begra'.

Material and methods

Plant material and phenotyping

Winter wheats 'Liwilla' and 'Begra' can be differentiated by their extremely different reactions to *Stagonospora nodorum* under controlled environment and field conditions (Arseniuk et al. 1995). The partially resistant 'Liwilla' and the susceptible 'Begra' were chosen for crossing and 111 DH lines were developed from anther cultures of the F_1 generation. DH lines and the two parents were inoculated with a mixture of 15 *S. nodorum* isolates originating from different geographic regions in Poland. The inoculum was produced using solid media containing 30 g agar/L and 33 g coarsely ground triticale seeds/L. Cultures of each isolate were incubated at 12 h light (300–400 nm) (22°C): 12 h dark (18°C). After two weeks, pycnidiospores were washed from the plates in distilled water and filtered through 2–3 layers of cheesecloth. The concentration of the spores per millilitre was calculated using a hematology counting camera. Wheat seedlings with a fully expanded fifth leaf (GS15 according to Zadoks et al. 1974) were sprayed with a pycnidiospore suspension (3×10^6 spores/mL). One drop of a surfactant (Tween 20) was added to each litre of the pathogen inoculum. After inoculation, the seedlings were left in a misting chamber for 96 h at a constant temperature of 22°C with weak illumination during the light period after which the misting system was turned off and the seedlings were allowed to dry. The photoperiod was set at 16 h light (22°C) : 8 h dark (17°C).

Three levels of resistance components were evaluated, including length of incubation period (days from inoculation to the first appearance of symptoms), length of latent period (days from inoculation to formation of pycnidia), and disease severity determined by percent leaf area with lesions (Cunfer et al. 1988; Jeger et al. 1983; Mullaney et al. 1982). After inoculation, the incubation period was checked every day for each fifth leaf. First symptoms of infection on wheat leaves were expressed as irregular chlorotic lesions. Fourteen days after inoculation, disease severity was scored using a 0–9 scale (0, immune, no symptoms or occasional hypersensitive fleck; scale from 1 to 9 corresponds to leaf necrosis coverage from 10 to 90%). To assess the latent period (checked every second day), after scoring disease severity, leaves were placed into plastic boxes with 150 ppm benzimidazole solution and high humidity was maintained during this period (Eyal et al. 1987). The analyses for the incubation period and the disease severity were conducted on an average of 13 seedlings of each DH line per repetition. Five leaves per repetition were used to assess the length of the latent period. The experiment was designed as a one-way experiment with two randomized blocks (repetitions). Owing to the limited space available in the controlled growth chamber, tests for each repetition were conducted separately. Analyses of variance and partial resistance components distribution were calculated by Statistica software version 5.0 (StatSoft Polska Sp., Kraków, Poland). The DH lines with the most extreme reaction for each partial resistance component were chosen for BSA according to the ranking revealed by the Newman–Keuls test and based upon consistency in reaction to the pathogen.

PCR and molecular markers

DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method from between five and eight seedlings of each DH line and parents with a fully developed fifth leaf (Saghai-Maroof et al. 1984). A TKO 100 Mini Fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.) was used to determine the DNA concentration. PCR amplifications were conducted in an 8-μL volume in a buffer containing $(NH_4)_2SO_4$ (Fermentas AB, Vilnius, Lithuania), 2.5 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, and dTTP, 0.5 μM of each primer, approximately 24 ng of template DNA, and 1 U *Taq* DNA polymerase (Fermentas). Eight-strip low-profile tubes (MJ Research Inc., Waltham, Mass.) were used. The UNOII 96 DNA thermal cycler (Biometra, Göttingen, Germany) was programmed for initial denaturation at 95°C for 60 s, 10 cycles of denaturation at 95°C for 30 s, annealing for 30 s (annealing temperature is provided below), and elongation at 72°C for 1–2 min (depending on expected PCR product length), followed by 30 cycles using a 90°C denaturation temperature. The final extension cycle at 72°C was extended to 11–12 min.

For BSA, sets of primers targeting microsatellites and resistance gene analogs were used. Of the 230 microsatellite primer sets published by Röder et al. (1998), 228 were used in our mapping project. To balance the number of

microsatellites among the 3 wheat genomes, 12 additional D genome microsatellites (gdm19, gdm33, gdm36, gdm46, gdm60, gdm61, gdm67, gdm84, gdm98, gdm108, gdm125, and gdm129 from Pestova et al. (2000*b*)) were used. In the BSA, 240 pairs of primers for microsatellite loci were amplified using annealing temperatures as described by Röder et al. (1998) and Pestova et al. (2000*b*). Additionally, a set of 20 primers (Table 1) targeting putative resistance gene analogs were chosen based upon the reports of Leister et al. (1996), Chen et al. (1998) and Collins et al. (1998). Resistance gene analogs were amplified by PCR using higher primer concentrations (4 μM) and the annealing temperature was set at 45°C (Chen et al. 1998).

All primers were synthesized by Sigma-Genosys (Cambridge, U.K.) and one primer of each pair was labeled at the 5′ end with one of the ABI dyes (6-FAM, HEX, or TET). After PCR, products of each sample labeled with 6-FAM, HEX, and TET dyes were mixed in a 1:2:1 proportion. From this mixture, 1.3 μL was added to 2.7 μL of loading mixture containing 0.3 μL GeneScan 500XL TAMRA internal size standard, 2 μL deionized formamide, and 0.4 μL of loading dye. The samples were mixed well and denatured at 95°C for 3 min and kept on ice until loading. Approximately 1 μL of each sample was loaded on AutoPAGE 4.25 or 4.5% w/v gels for microsatellites or resistance gene analog products, respectively (Sigma-Aldrich, Poznań, Poland). PCR products were electrophoresed and detected with an ABI PRISM 377XL DNA sequencer supplied with Genscan software (Applied Biosystems (formerly PE Biosystems), Foster City, Calif.).

To provide additional molecular markers in certain regions of the wheat genome potentially linked to QTLs, microsatellites WMC257, WMC262, and WMC265 were used (Varshney et al. 2000). Another 33 microsatellites (BARC prefix) localized on chromosomes 2B, 3B, 5B, and 5D were chosen from the Web site (www.scabusa.org/pdfs/BARC_SSRs_011101.html). Sequence information for particular restriction fragment length polymorphism (RFLP) probes already mapped to regions of interest was acquired through sequencing both ends of the probe (bcd1119, bcd1140, bcd445, bcd907, cdo412, cdo57, cdo684, ksuD30, ksuG53, MWG52) or from sequence information deposited in the Graingenes database (www.graingenes.org) (abg473, BE438868, BE438939, BE439130, BE439133, U36894). To convert a probe to a sequence-tagged site (STS) marker, at least one pair of primers was designed for each sequence with GeneFisher version 1.3 software (http://bibiserv.techfak.uni-bielefeld.de/genefisher/) and synthesized by Annovis (Annovis Inc. Aston, Pa.). For newly synthesized primers, the annealing temperature for PCR was determined using a Mastercycler Gradient (Brinkmann Instruments Inc., Westbury, N.Y.). Monomorphic STS fragments were digested with a set of nine restriction endonucleases to generate cleaved amplified polymorphic sequence (CAPS) markers as described by Konieczny and Ausubel (1993). The four-base cutter restriction enzymes *Alu*I, *Mse*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*I, *Mbo*I, *Rsa*I, and *Taq*I were used according to the manufacturer's instructions (Invitrogen, Carlsbad, Calif.). DNA fragments originated from STS and CAPS markers were resolved using 1.8–2.0% GTG SeaKem agarose or 2.25–3.0% Metaphor agarose (when better resolution was required) (BioWhittaker Molecular Applications, Rockland, Maine), 0.5× TBE buffer (90 mM Tris, 90 mM borate, 2 mM EDTA (pH 8.0)), 1× TBE in gel at 4–5 V/cm for 4–5 h. Gels were stained with ethidium bromide or with SYBR Gold (Molecular Probes, Eugene, Oreg.) (when higher sensitivity was required) and photographed on a transilluminator.

Bulked segregant analysis and QTL mapping

Based on the phenotyping experiments, individuals with the highest and lowest levels of the three resistance components were chosen for BSA. For each component assessed, five susceptible and five resistant DH lines were chosen to establish alternative bulks by mixing equal amounts of DNA of each line. Therefore, three sets of bulks (for each component) and two parental cultivars were initially screened. Only one polymorphic pattern was considered, which was when the DNA band detected in the resistant parent 'Liwilla' was also detected in the resistant bulk and was not present in both DNA profiles of the susceptible parent 'Begra' and the susceptible bulk. Nevertheless, despite clear polymorphisms between parents and between bulks, an intensity polymorphism between bulks was also evaluated as described by Chantret et al. (2000). In this case, the DNA band detected in the resistant parent was present in both bulks, but in the susceptible bulk this specific PCR product was faint. When a suitable polymorphism was detected between parents and bulks, the particular primer pair was tested on a set of 12 DNA samples consisting of the two parents and 10 DH lines used to establish alternative bulks. Further selection of molecular markers was based on the correspondence between the phenotypes of the 10 DH lines (determined in the experiments conducted under controlled environment) and their corresponding molecular phenotype (defined by microsatellite or putative resistance gene analog fragment). If the disease reaction and molecular phenotype were in agreement for at least eight of the 10 DH lines, the molecular marker was tested on the whole segregating population.

The BSA method allowed us to identify markers putatively linked to QTLs that controlled resistance components. To verify this hypothesis, segregation data of each marker and each of the three components of partial resistance were analyzed using an interval mapping method (MapQTL 4.0 software, Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands). The minimal LOD value for this initial analysis was 1.5. Markers with this minimum LOD value were used to construct linkage groups using JoinMap version 2.0 software (Centre for Plant Breeding and Reproduction Research). The resistance gene analogs with unknown location in the wheat genome were mapped using a set of *T. aestivum* 'Chinese Spring' ditelosomic lines kindly provided by A. Lukaszewski (University of California, Riverside, Calif.). Established linkage groups were used for QTL mapping using the interval mapping method with a LOD threshold of 3.0.

Results

Phenotyping

Parental varieties and offspring lines that differed significantly for each component were analyzed (Table 2). A rela-

Table 1. Primer sets used to identify putative resistance genes analogs (RGAs) in wheat.

Set No.	Forward primer	Sequence (5′→3′)	Reverse primer	Sequence (5′→3′)
1	S2	GGIGGIGTIGGIAAIACIAC	AS2	IAAIGCIAGIGGIAAICC
2	S2	GGIGGIGTIGGIAAIACIAC	AS3	IAGIGCIAGIGGIAGICC
3	Kinase2D	CTACTGNTNCTNGACGACGT	AS2	IAAIGCIAGIGGIAAICC
4	Kinase2E	CTACTGNTNCTNGACGATGT	AS2	IAAIGCIAGIGGIAAICC
5	Kinase2F	CTACTGNTNCTNGATGACGT	AS2	IAAIGCIAGIGGIAAICC
6	Kinase2G	CTACTGNTNCTNGATGATGT	AS2	IAAIGCIAGIGGIAAICC
7	Kinase2D	CTACTGNTNCTNGACGACGT	AS3	IAGIGCIAGIGGIAGICC
8	Kinase2E	CTACTGNTNCTNGACGATGT	AS3	IAGIGCIAGIGGIAGICC
9	Kinase2F	CTACTGNTNCTNGATGACGT	AS3	IAGIGCIAGIGGIAGICC
10	Kinase2G	CTACTGNTNCTNGATGATGT	AS3	IAGIGCIAGIGGIAGICC
11	S2	GGIGGIGTIGGIAAIACIAC	MHD1	CGACAGTCNATCATGCAT
12	S2	GGIGGIGTIGGIAAIACIAC	MHD2	CGACAGTCNATCGTGCAT
13	S2	GGIGGIGTIGGIAAIACIAC	MHD3	CGACAGTCNGTCATGCAT
14	S2	GGIGGIGTIGGIAAIACIAC	MHD4	CGACAGTCNGTCGTGCAT
15	PtoKin1	GCATTGGAACAAGGTGAA	PtoKin2	AGGGGGACCACCACGTAG
16	RLRR	CGCAACCACTAGAGTAAC	RLRR	ACACTGGTCCATGAGGTT
17	XLRR	CCGTTGGACAGGAAGGAG	XLRR	CCCATAGACCGGACTGTT
18	NLRR	TAGGGCCTCTTGCATCGT	NLRR	TATAAAAAGTGCCGGACT
19	CLRR	TTTTCGTGTTCAACGACG	CLRR	TAACGTCTATCGACTTCT
20	NBS	GGAATGGGNGGNGTNGGNAARAC	NBS	YCTAGTTGTRAYDATDAYYYTRC

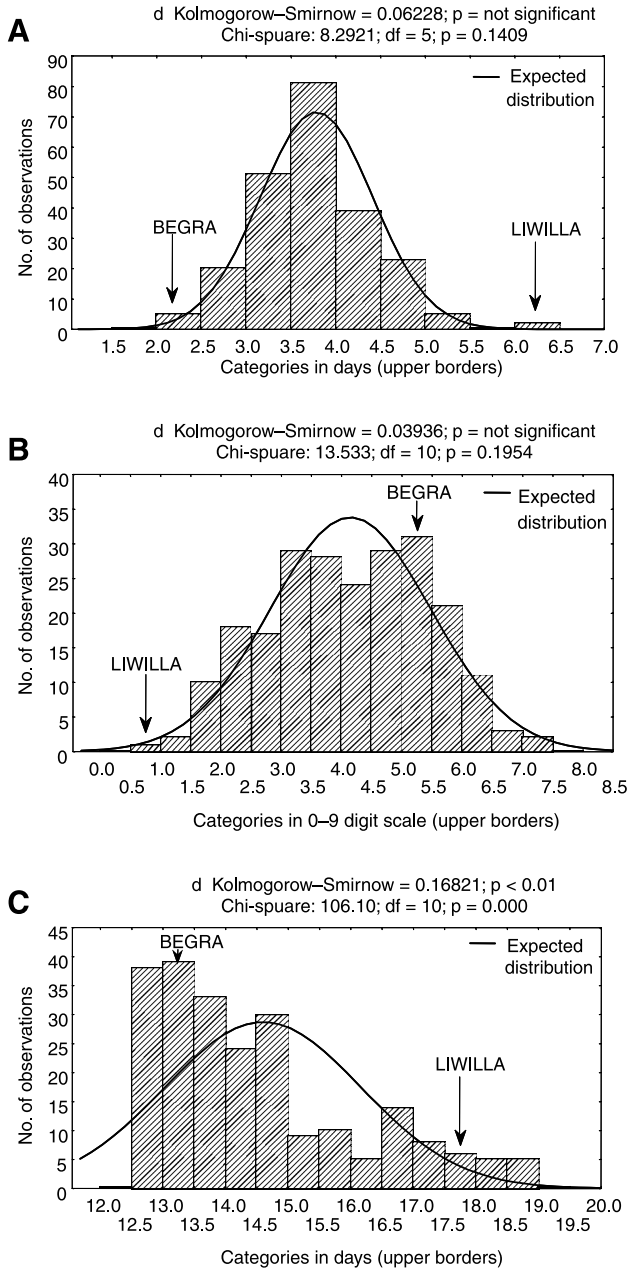
Note: I, inosine; R, A or G; Y, C or T; D, A or G or T; N, A or G or C or T.

Table 2. Analysis of variance for partial resistance components of resistant parent ‘Liwilla’, susceptible parent ‘Begra’, and offspring DH lines at the fifth leaf stage to *Stagonospora nodorum.*

(*a*) Variable: incubation period						
Source	df		MS	*F* value		*P* value
Blocks	1		6.715	33.980		<0.001
Lines	112		0.541	2.735		<0.001
Residual	112		0.198			
From ANOVA	Parents		Offspring DH Lines			
	Liwilla	Begra	Min.	Mean	Max.	SD
	5.5	2.6	2.7	3.8	5.2	0.63
(*b*) Variable: disease severity						
Source	df		MS	*F* value		*P* value
Blocks	1		15.924	28.644		<0.001
Lines	112		2.872	51.67		<0.001
Residual	112		0.556			
From ANOVA	Parents		Offspring DH Lines			
	Liwilla	Begra	Min.	Mean	Max.	SD
	1.3	5.1	1.7	4.2	6.7	1.33
(*c*) Variable: latent period						
Source	df		MS	*F* value		*P* value
Blocks	1		8.200	8.551		0.004
Lines	112		3.918	4.086		<0.001
Residual	112		0.959			
From ANOVA	Parents		Offspring DH Lines			
	Liwilla	Begra	Min.	Mean	Max.	SD
	17.5	13.4	13.0	14.6	18.4	1.57

tively broad range of variation was found for disease severity and the latent period, and a lower range of variation was found for the incubation period. The DH lines showed continuous variation for each component with normal distribution for the incubation period and disease severity (Figs. 1A and 1B). In contrast, the latent period was not normally distributed (Fig. 1C). Three DH lines significantly transgressed the parental genotype ‘Begra’ in susceptibility. The reaction of the DH lines as measured by length of latent period for some DH lines were outside the range of parents, but statistically significant transgressive segregation was not detected. We found a high positive correlation (0.79, $P <$

Fig. 1. Distribution of 111 DH lines, resistant parent 'Liwilla', and susceptible parent 'Begra' (all together 226 observations) for incubation period (A), disease severity (B), and latent period (C) and their fit to a normal distribution as tested by the Kolmogorow–Smirnow test.



0.01) between the latent and incubation periods and negative correlations between disease severity and either the latent period (–0.83, $P < 0.01$) or the incubation period (–0.78, $P < 0.01$).

Molecular markers

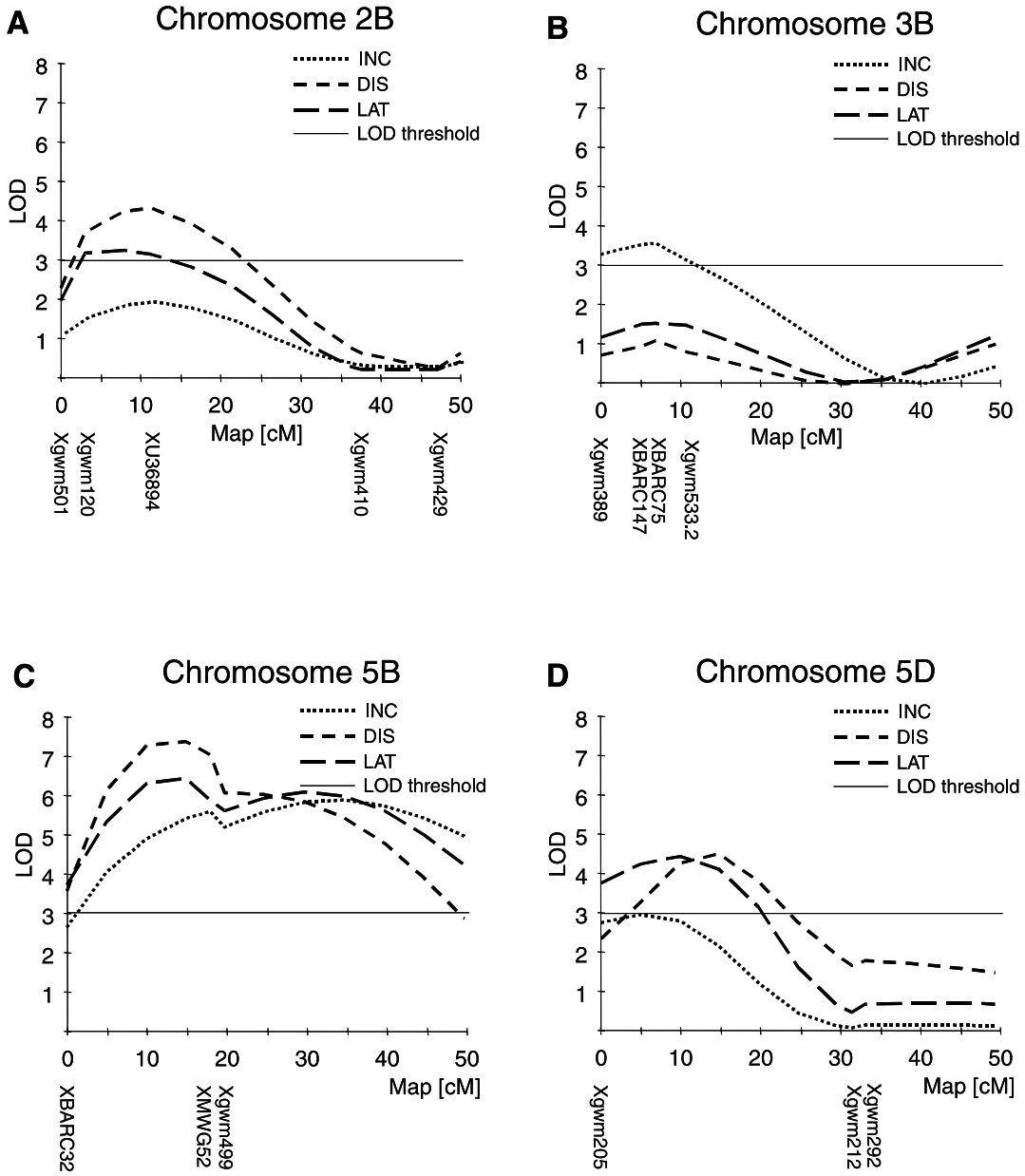
Only six sets of primers targeting putative resistance gene analogs produced polymorphic PCR products for 10 different loci. The 240 pairs of primers used in this study could potentially amplify 291 microsatellite loci as reported by Pestova et al. (2000*b*) and Röder et al. (1998). We detected 245 microsatellite loci where 124 were polymorphic between the parents and the remaining were monomorphic. Only 34 microsatellite loci were polymorphic both between parents and between bulks within at least one of the three sets of bulks established for each resistance component. Only one marker, gwm499, showed clear polymorphism between bulks of each set and between parents. The other 33 showed polymorphism between parents and an intensity polymorphism between bulks. The microsatellite gwm499 unequivocally distinguished the five resistant from the five susceptible DH lines used to establish alternative bulks. In the case of the other 33 markers, only 15 markers showed agreement between phenotype and molecular marker phenotype for at least 8 of 10 DH lines. However, only six markers had a LOD value greater than 1.5 after initial QTL searching. These markers were located on chromosomes 2B (Ptokin.1, gwm120), 3B (gwm389, gwm533.2), 5B (gwm499), and 5D (gwm212). The markers, along with a number of additional polymorphic markers, were used to further analyze chromosomes 2B (gwm257, gwm410, gwm429, gwm501, BARC128, BARC200, U36894), 3B (BARC68, BARC75, BARC147), 5B (abg473, BARC11, BARC32, MWG52, WMC262, XLRR.2), and 5D (gwm174, gwm205, gwm271, gwm292). Markers abg473 (amplified in PCR by primers 5′-TGAGCTAGTAGAGAAAAGGCCAAG-3′ and 5′-CAGTACCTACCTTGGCAAATTAGG-3′) and MWG52 (amplified in PCR by primers 5′-ATGCCCCAGCATTTTGTTCAG-3′ and 5′-GTACAAGGTCAGTCTGTGAGG-3′) revealed polymorphism after digestion with the *Taq*I restriction enzyme. The other STS markers were not polymorphic or the primers did not amplify. After analysis of segregation data based upon on the expected 1:1 segregation ratio, the following markers were eliminated from the data set ($P < 0.05$): Ptokin.1 ($\chi^2 = 12.33$), BARC128 ($\chi^2 = 8.66$), XLRR.2 ($\chi^2 = 9.81$), abg473 ($\chi^2 = 7.58$), WMC262 ($\chi^2 = 13.13$), and gwm174 ($\chi^2 = 8.82$).

QTL analysis

Four QTLs associated with partial resistance components were derived from 'Liwilla'. These QTLs were mapped to chromosomes 2B, 3B, 5B, and 5D (Fig. 2). The QTLs detected on chromosomes 5B and 5D affected all three of the partial resistance components. The QTL on chromosome 2B affected both disease severity and latent period. In general, the LOD traces for each of the three traits were similar, indicating that the same gene or a tightly linked gene was associated with variation in each of the three components of resistance.

The percentage of phenotypic variance explained by a single QTL ranged from 14 to 21% for the incubation period, from 16 to 37% for disease severity, and from 13 to 28% for the latent period. The QTL mapping to chromosome 2B contributed moderately to low disease severity (16%) and a long latent period (13%) and was located on the proximal part of the long arm of the chromosome (Fig. 2A). The QTL located on the distal part of the short arm of chromosome 3B was only significantly associated with long incubation period and explained 14% of the phenotypic variance of the trait (Fig. 2B). The QTL detected on chromosome 5B had the highest LOD values ranging from 5.57 to 7.38 for each partial resistance (Fig. 2C). This major QTL explained 21, 30,

Fig. 2. LOD curves based on interval mapping of QTLs for long incubation period (INC), low disease severity (DIS), and long latent period (LAT) on linkage groups corresponding to wheat chromosomes 2B (A), 3B (B), 5B (C) and 5D (D).



and 27% of the phenotypic variance for the incubation period, disease severity, and the latent period, respectively. The QTL detected on chromosome 5D had a major effect on incubation period, disease severity, and latent period and explained phenotypic variance of 17, 37, and 28%, respectively. However, this QTL was associated with incubation period declaring LOD value 2.94, slightly below the LOD threshold of 3.0 (Fig. 2D).

Discussion

QTL analysis of *Stagonospora nodorum* blotch resistance at the fifth leaf stage of winter wheat 'Liwilla' revealed four regions in the wheat genome associated with resistance and is in agreement with other studies postulating a concept of resistance to the disease conditioned by several genes (Nelson and Marshall 1990). We found four QTLs significantly associated with partial resistance components located on chromosomes 2B, 3B, 5B, and 5D. These chromosomes were already shown to be involved in disease resistance studies using monosomic lines (Auriau et al. 1988; Hu et al. 1996; Waląg and Dzięgło 1985, 1987) and chromosomal substitution lines (Kleijer et al. 1980; Nicholson et al. 1993). Partial resistance of line L22 to *Stagonospora nodorum* blotch studied by Auriau et al. (1988) revealed six and nine chromosomes to be implicated in longer incubation periods and lower disease intensity, respectively. Among them, chromosomes 2B and 5B were specified, but chromosome 3B was found to be involved in a susceptible reaction regarding incubation period. In our work, the QTL on chromo-

some 5B had the highest LOD score for each resistance component studied. These results are contrary to the findings of Kleijer (1977) and Waląg and Dzięgło (1985) who postulated that strong resistance inhibition was present on chromosome 5B. In the same segment of chromosome 5D, delimited by the two loci *Xgwm205* and *Xgwm212*, our QTL associated with a single gene, *Srb3*, conferring resistance to *S. nodorum* (Ellerbrook et al. 1999). However, our mapping region on 5D is large (over 30 cM) and has low marker density, so we can only speculate about any relation between these resistance loci. To our knowledge, there is one study exploring resistance in *T. spelta* to *S. nodorum* using QTL analysis (Messmer et al. 1997). Its mapping population (226 recombinant inbred lines) was derived from a wide cross between *T. spelta* 'Oberkulmer' and *T. aestivum* 'Forno'. Disease severity on ears and leaves was assessed in field experiments after artificial inoculation with *S. nodorum*. In total, up to seven QTLs were mapped for resistance to leaf infection, which explained 5–13% of the phenotypic variation, but only two were consistent over 3 years of experiments. Jointly, these QTLs could explain 36% of the variation observed for leaf blotch. The total variation explained by these QTLs is much lower than that determined in our study, where a single QTL could explain 13–37% of the phenotypic variation. Nevertheless, some portions of variation in the three resistance components remain unexplained. This could be due to QTLs that remain undetected or because no markers have been identified in regions associated with those QTLs.

The partial resistance components investigated in our study were highly correlated as reported by some investigators (Cunfer et al. 1988; Ecker et al. 1989; Mullaney et al. 1982). These highly significant correlations between components suggest partial pleiotropy or linkage (Ecker et al. 1989). This conclusion is also supported by similar LOD traces calculated for each component on the four chromosomes investigated.

The use of microsatellites coupled with BSA is probably the most convenient way to map QTLs in wheat. This approach was applied in tagging single resistance genes to *S. nodorum* in *T. tauschii* (Murphy et al. 1999) and durum wheat (Cao et al. 2001). We confirm usefulness of this approach for mapping quantitative resistance loci to this pathogen in wheat. Moreover, we showed that even frequently observed intensity polymorphism between bulks could be useful in QTL analyses. The weight of the four QTLs associated with partial resistance components found with BSA was assessed at being between 13 and 37%, similar to that detected for other pathogens in wheat (Bai at al. 1999; Chantret et al. 2000; William et al. 1997). Further molecular analyses are needed to enrich QTL regions with additional markers for better resolution. The two QTLs with major effects detected on chromosomes 5B and 5D could be transferred to different genetic backgrounds by marker-assisted selection to elevate their effectiveness in conditioning resistance to *Stagonospora nodorum* blotch.

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